

# CLAIMS

1. A composition for identifying a nucleotide at a predetermined position of a target polynucleotide in a sample, said composition comprising:

(a) an oligonucleotide primer comprising a first sequence which hybridizes to said target polynucleotide immediately 3' of said nucleotide, and a second sequence which does not hybridize to said target polynucleotide in the presence of a third sequence; and

(b) an oligonucleotide probe comprising said third sequence which hybridizes to said second sequence of said oligonucleotide primer, said oligonucleotide probe labeled with a first member of a pair of interactive labels.

2. The composition of claim 1, further comprising a first polynucleotide chain terminator, which is incorporated in a template-dependent manner into said oligonucleotide primer by a polynucleotide synthesis enzyme.

3. The composition of claim 2, further comprising one or more of a second, a third and/or a fourth polynucleotide chain terminator, wherein said first, second, third and fourth polynucleotide terminators are not identical.

4. The composition of claim 2, wherein said first polynucleotide chain terminator is labeled with a second member of said pair of interactive labels.

5. The composition of claim 4, wherein said first and second members of said pair of interactive labels interact with each other to generate a signal by fluorescent resonance energy transfer.

6. The composition of claim 1, further comprising a template-dependent polynucleotide synthesis enzyme for incorporating in a template-dependent manner a complementary polynucleotide chain terminator into said oligonucleotide primer.

7. The composition of claim 6, wherein said polynucleotide synthesis enzyme is a JDF-3 DNA polymerase.

8. The composition of claim 2, wherein said oligonucleotide primer comprises a separation moiety that permits separation of said oligonucleotide primer and/or said oligonucleotide probe hybridized to said primer from unincorporated polynucleotide chain terminator, and oligonucleotide probe which is not hybridized to said oligonucleotide primer.

5 9. The composition of claim 8, further comprising a target moiety specific for said separation moiety, wherein said separation moiety binds to said target moiety to permit said separation.

10. The composition of claim 9, wherein said target moiety is attached to a solid support.

10 11. The composition of claim 4, wherein said first and second members of said pair of interactive labels are fluorescent molecules which interact with each other to generate a signal by fluorescent resonance energy transfer.

12. A composition for identifying a nucleotide at a predetermined position of a target polynucleotide in a sample, said composition comprising:

15 (a) an oligonucleotide primer comprising a first sequence which hybridizes to the target polynucleotide immediately 3' of said nucleotide, and is covalently attached to a tag molecule; and

(b) an anti-tag molecule which binds to said tag molecule, said anti-tag molecule labeled with a first member of a pair of interactive labels.

20 13. The composition of claim 12, wherein said tag molecule is located on the 5' terminal of said oligonucleotide primer.

14. The composition of claim 13, wherein said tag molecule is a first member of a specific binding pair which comprises said first member and a second member.

15. The composition of claim 14, wherein said anti-tag molecule is said second member of said specific binding pair.

16. The composition of claim 15, wherein said specific binding pair is a biotin-streptavidin pair.

17. The composition of claim 1, wherein said second sequence is at the 5' terminal of said first sequence.

5 18. The composition of claim 1, further comprising a labeled conventional deoxynucleotide, and the other three unlabeled chain terminators, wherein said labeled conventional deoxynucleotide is incorporated into the oligonucleotide primer at a position corresponding to the predetermined nucleotide of the target polynucleotide.

10 19. The composition of claim 1, wherein one member of the pair of interactive labels is a quencher molecule.

20. A kit for identifying a nucleotide at a predetermined position of a target polynucleotide in a sample, said kit comprising:

15 (a) an oligonucleotide primer comprising a first sequence which hybridizes to said target polynucleotide immediately 3' of said nucleotide, and a second sequence which does not hybridize to said target polynucleotide in the presence of a third sequence;

(b) an oligonucleotide probe comprising said third sequence which hybridizes to said second sequence of said oligonucleotide primer, said oligonucleotide probe labeled with a first member of a pair of interactive labels; and

(c) packaging materials therefore.

20 21. The kit of claim 20, further comprising a polynucleotide chain terminator, which can be incorporated in a template-dependent manner into said oligonucleotide primer by a polynucleotide synthesis enzyme.

25 22. The kit of claim 21, further comprising one or more of a second, a third and/or a fourth polynucleotide chain terminator, wherein said first, second, third and fourth polynucleotide terminators are not identical.

23. The kit of claim 21, wherein said polynucleotide chain terminator is labeled with a second member of said pair of interactive labels.

24. The kit of claim 20, further comprising a template-dependent polynucleotide synthesis enzyme for incorporating in a template-dependent manner a complementary polynucleotide chain terminator into said oligonucleotide primer.

25. The kit of claim 24, wherein said polynucleotide synthesis enzyme is a JDF-3 DNA polymerase.

26. A kit for identifying a nucleotide at a predetermined position of a target polynucleotide in a sample, said kit comprising:

(a) an oligonucleotide primer comprising a first sequence which hybridizes to the target polynucleotide immediately 3' of said nucleotide, and is covalently attached to a tag molecule;

(b) an anti-tag molecule which binds to said tag molecule, said anti-tag molecule being labeled with a first member of a pair of interactive labels; and

(c) packaging materials therefore.

27. The kit of claim 26, wherein said tag molecule is a first member of a specific binding pair which comprises said first member and a second member.

28. The kit of claim 27, wherein said anti-tag molecule is said second member of said specific binding pair.

29. The kit of claim 28, wherein said specific binding pair comprises a biotin-streptavidin pair.

30. A method of identifying the presence of a nucleotide at a predetermined position of a target polynucleotide, said method comprising:

(a) incubating said target polynucleotide in a reaction mixture comprising an oligonucleotide primer which hybridizes to said target polynucleotide immediately 3' of said nucleotide, an

oligonucleotide probe which hybridizes to said oligonucleotide primer and labeled with a first member of a pair of interactive labels, a polynucleotide chain terminator labeled with a second member of said pair of interactive labels, wherein said incubating permits said polynucleotide chain terminator to be incorporated into said oligonucleotide primer, and  
 5 permits said oligonucleotide probe to hybridize to said oligonucleotide primer to permit said pair of interactive labels to generate a signal; and

(b) detecting said signal, wherein said detection is indicative of the presence of said nucleotide in said target polynucleotide.

31. A method of identifying the presence of a nucleotide at a predetermined position of a  
 10 target polynucleotide, said method comprising the steps:

(a) incubating said target polynucleotide in a reaction mixture comprising an oligonucleotide primer which hybridizes to said target polynucleotide immediately 3' of said nucleotide and a polynucleotide chain terminator labeled with a second member of a pair of interactive labels, wherein said incubating permits said polynucleotide chain terminator to be incorporated into  
 15 said oligonucleotide primer;

(b) incubating the oligonucleotide primer comprising said second member of said pair of interactive labels with an oligonucleotide probe labeled with a first member of said pair of interactive labels, such that formation of a hybrid between said oligonucleotide probe and said primer permits said pair of interactive labels to generate a signal; and

20 (c) detecting said signal, wherein said detection is indicative of the presence of said nucleotide in said target polynucleotide.

32. The method of claim 30 or 31, wherein said signal is generated by fluorescent resonance energy transfer.

33. The method of claim 30 or 31, wherein said oligonucleotide primer comprises a first  
 25 sequence which hybridizes to said target polynucleotide and a second sequence which does not hybridize to said target polynucleotide in the presence of a third sequence.

34. The method of claim 33, wherein said oligonucleotide probe comprises said third sequence which hybridizes to said second sequence of said oligonucleotide primer.

35. The method of claim 30 or 31, wherein said polynucleotide chain terminator is incorporated by a polynucleotide synthesis enzyme.

5 36. The method of claim 30 or 31, wherein said reaction mixture further comprises one or more of a second, a third and/or a fourth polynucleotide chain terminator, wherein said first, second, third and fourth polynucleotide terminators are not identical.

37. The method of claim 35, wherein said polynucleotide synthesis enzyme is a JDF-3 DNA polymerase.

10 38. The method of claim 33, wherein said second sequence is at the 5' terminal of said first sequence.

39. The method of claim 30 or 31, wherein said oligonucleotide primer comprises a separation moiety that permits separation of said oligonucleotide primer from said reaction mixture.

15 40. The method of claim 39, wherein a target moiety is provided for said separation moiety to form a specific binding pair for separation.

41. The method of claim 40, wherein said target moiety is attached to a solid support.

42. A method of identifying the presence of a nucleotide at a predetermined position of a target polynucleotide, said method comprising:

20 (a) incubating said target polynucleotide in a reaction mixture comprising an anti-tag molecule labeled with a first member of a pair of interactive labels, a polynucleotide chain terminator labeled with a second member of said pair of interactive labels, and an oligonucleotide primer which hybridizes to said target polynucleotide immediately 3' of the nucleotide, said oligonucleotide primer covalently coupled to a tag molecule, wherein said  
25 incubating permits said polynucleotide chain terminator to be incorporated into said oligonucleotide primer, and said incubating also permits said anti-tag molecule to interact

with said tag molecule on said oligonucleotide primer, so that said pair of interactive labels generate a signal; and

(b) detecting said signal, wherein said detection is indicative of the presence of said nucleotide in said target polynucleotide.

5 43. The method of claim 42, wherein said signal is generated by fluorescent resonance energy transfer.

44. The method of claim 41, wherein said tag molecule is at 5' terminal of said oligonucleotide primer.

10 45. The method of claim 44, wherein said tag molecule comprises a first member of a specific binding pair which comprises said first member and a second member.

46. The method of claim 45, wherein said anti-tag molecule comprises said second member of said specific binding pair.

47. The method of claim 46, wherein said specific binding pair is a biotin-streptavidin binding pair.

15 48. The method of claim 30 or 31, wherein one member of the pair of interactive labels is a quencher molecule.

49. The method of claim 30, 31, or 42, wherein said chain terminator is one selected from the group consisting of: a dideoxynucleotide triphosphate, a ribofuranose analog, a reversible nucleotide terminator, and an acyclic terminator.

20 50. The method of claim 30, 31, or 42, wherein the target polynucleotide presents in a sample.

51. A method of identifying the presence of a nucleotide at a predetermined position of a target polynucleotide, said method comprising:

25 (a) incubating said target polynucleotide in a reaction mixture comprising an oligonucleotide primer which hybridizes to said target polynucleotide immediately 3' of said nucleotide, an

oligonucleotide probe which hybridizes to said oligonucleotide primer and labeled with a first member of a pair of interactive labels, a conventional deoxynucleotide labeled with a second member of said pair of interactive labels, wherein said incubating permits said labeled conventional deoxynucleotide to be incorporated into said oligonucleotide primer at a position  
 5 corresponding to the predetermined position of the target polynucleotide, and permits said oligonucleotide probe to hybridize to said oligonucleotide primer to permit said pair of interactive labels to generate a signal; and

(b) detecting said signal, wherein said detection is indicative of the presence of said nucleotide in said target polynucleotide.

10 52. A method of identifying the presence of a nucleotide at a predetermined position of a target polynucleotide, said method comprising the steps:

(a) incubating said target polynucleotide in a reaction mixture comprising an oligonucleotide primer which hybridizes to said target polynucleotide immediately 3' of said nucleotide and a conventional deoxynucleotide labeled with a second member of a pair of interactive labels,  
 15 wherein said incubating permits said conventional deoxynucleotide to be incorporated into said oligonucleotide primer at a position corresponding to the predetermined position of the target polynucleotide;

(b) incubating the oligonucleotide primer comprising said second member of said pair of interactive labels with an oligonucleotide probe labeled with a first member of said pair of  
 20 interactive labels, such that formation of a hybrid between said oligonucleotide probe and said primer permits said pair of interactive labels to generate a signal; and

(c) detecting said signal, wherein said detection is indicative of the presence of said nucleotide in said target polynucleotide.

25 53. A method of identifying the presence of a nucleotide at a predetermined position of a target polynucleotide, said method comprising:

(a) incubating said target polynucleotide in a reaction mixture comprising an anti-tag molecule labeled with a first member of a pair of interactive labels, a conventional



deoxynucleotide labeled with a second member of said pair of interactive labels, and an oligonucleotide primer which hybridizes to said target polynucleotide immediately 3' of the nucleotide, said oligonucleotide primer covalently coupled to a tag molecule, wherein said incubating permits said conventional deoxynucleotide to be incorporated into said

5 oligonucleotide primer at a position corresponding to the predetermined position of the target polynucleotide, and said incubating also permits said anti-tag molecule to interact with said tag molecule on said oligonucleotide primer, so that said pair of interactive labels generate a signal; and

(b) detecting said signal, wherein said detection is indicative of the presence of said  
10 nucleotide in said target polynucleotide.

54. The method of claim 51, 52, or 53, wherein the reaction mixture further comprising at least one unlabeled chain terminator.

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